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Research Article

Nutritional Improvement and Methane Gas Reduction of Poor Quality Roughages through *Saccharomyces cerevisiae* Fermentation in Sheep Feeding *In-vitro*

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ABSTRACT

This research was carried out the nutritive improvement of low quality roughages using yeast (*S. cerevisiae*) fermentation. Rice straw and Sugarcane bagasse were used as roughage substrate while the roughage mixture was made by rice straw and sugarcane bagasse at 1:3 ratio. Feeds were fermented with 0, 1, 3, 5, and 7% yeast for 5 days of incubation period. Results found that yeast fermentation increased the CP, TA, and NFE content of rice straw by 39.26, 7.49, and 13.53% respectively while reduced CF, EE, and ME by 24.45, 29.75, and 17.30% respectively, than non-fermented straw. Yeast fermentation increased 25.28, 38.72 and 31.25% TP, Ca and P content of the fermented straw. In *in-vitro* method result showed that The CH_4 gas production decreased 8.78% in fermented straw. Maximum CP, TA, and NFE content of Sugarcane bagasse increased by 78.40, 48.60, and 14.77% respectively, and reducing CF, EE, and ME by 25.15, 62.5, and 13.86% than the control bagasse. Result showed that TP, Ca and P content of fermented bagasse increased by 38.70, 42.0 and 28.57% respectively and decline 39.97% CH_4 gas production *in-vitro* trial. Fermented mixed roughage (Rice straw: Sugarcane bagasse; (1: 3) which was increased by 29.91% CP, 21.12% TA, and 16.91% NFE content and decreased by 26.71% CF, 34.61% EE, and 13.75% ME. Fermentation increased 24.56% TP content of roughage mixture. The CH_4 gas production decreased 46.56% of fermented roughage mixture. The results showed that fermentation improved the TP, Ca and P content of the roughage mixture by 24.56, 35.53 and 34.0% respectively. After completing the research work the results found that nutritive value of poor quality roughages were increased significantly through *S. cerevisiae* fermentation.

Introduction

According to Ross et al., (2002), fermentation is one of the oldest methods of food processing and preservation. It can also enhance the nutritional qualities of the original product (Frias et al., 2008).Yeast fermentation aids in the breakdown of feed's nutrients, making them simpler to digest. The conversion of carbohydrates like starch and sugar into alcohol or acids is a process known as fermentation. Fermented foods get a unique zest and sourness from these alcohols or acids, which also serve as a natural preservative. Beneficial bacterial growth is encouraged during fermentation. As carbohydrates are ingested, protein levels are either boosted by the buildup of microbial biomass or by the concentration of protein already present in the substrate (Day & Morawicki, 2018). The digestive systems of ruminants frequently utilize yeast as a probiotic and prebiotic.When forages are scarce, rice straws and bagasse have the potential to be used as animal feed. These have little nutritional value and are low in protein, palatability, and digestibility. Lignocellulosic components are found in bagasse and rice straws. The degree of lignification and the presence of crystalline cellulose serve as a steric barrier to stop ruminal microbes from enzymatically attacking cellulose and hemicellulose. Microbial fermentation, and adding enzymes are biological therapies (Doyle et al., 1986). The use of rice straw by ruminants has been improved through the investigation of several strategies. The most successful method is the microbial treatment, which uses extracellular ligninolytic enzymes or specialized enzymes that break down cellulose or hemicellulose to increase the nutritional content of rice

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straw. The cost-effective and more practical method to increase the nutritional content of rice straw is to treat it with fungi or enzymes (Sarnklong et al., 2010). Sugarcane bagasse is the principal agro-industrial byproduct of sugarcane processing (Costa et al., 2015). Although sugarcane bagasse is recognized as a low-quality roughage due to its 40% digestible fiber level and low crude protein (CP) content, it has the advantages of being readily available when fodder is in short supply and being more affordable than other common sources of roughage (Barros, 2010). Approximately 50% cellulose, 25% hemicellulose, and 25% lignin are present in sugarcane bagasse (Pandey et al., 2000). With less than 3% crude protein, (40–45%) cellulose, (28–30%) hemicellulose, and (19–21%) lignin, it has a high fiber content but little protein (Costa et al., 2015). Sugarcane bagasse can be recycled to create products with additional value such enzymes, amino acids, organic acids, and substances with medicinal use (Pandey et al., 2000). According to Marjuki, (2013), chemical pre-treatment prior to fermentation, such as ammoniation, can optimize microbial activity. The best characteristics of sugarcane bagasse silage led to the fermentation of sugarcane bagasse by *L. casei* TH14 in conjunction with cellulase and molasses (So et al., 2020). In order to increase the bioavailability of nutrient and decrease anti-nutritional factors, fermentation can enhance the nutritional quality of animal feed (Song et al., 2010). Solid state fermentation would be an excellent biotechnological approach for better utilizing a significant amount of crop residues to improve the nutritional values. With this hypothesis, following aims were taken to reach the goals.

Objectives

Nutritional upgradation of poor quality roughages (rice straw and sugarcane bagasse) through yeast (*S.cerevisiae*) fermentation.

Methodology

Different levels of yeast (0, 1, 3 and 5%) on selective roughages (Rice straw and Sugarcane bagasse) were allowed for fermentation for five (5) days. The work was done by following steps.

Collection of substrates for fermentation inoculants

Rice straw and sugarcane bagasse were used as roughage source. Rice straw was collected from the BADC (Bangladesh Agriculture Development Corporation); DokkhinShurma, Sylhet and sugarcane bagasse was collected from road side sugarcane juice shop, Amborkhana, Sylhet city corporation, Sylhet. Rice straw and sugarcane bagasse were crushed by crusher machine (Model 9F-40, China). Commercial dry yeast (*S. cerevisae, strain CY203*) was collected from Mega grocery shop, Sylhet. Commercial dry yeast (*Saccharomyces cerevisae, strain CY203*), molasses and warm water (39ºC) were added with substrate allowing for fermentation for 5 days.

Fermentation of rice straw and sugarcane bagasse

Fermentation was carried out with the mixture of 50 g rice straw, 50 ml warm water (100% of substrate) and dry yeast (0, 1, 3, 5, and 7%) in a polythene bag. The incubation periods of all samples were 5 days at room temparature. The treatments were T_0,T_1 T_2, T_3 and T_4

Fermentation of sugarcane bagasse

Fermentation was carried out with the mixture of 50 g sugarcane bagasse, 50 ml warm water (100% of substrate) and dry yeast (0, 1, 3, 5, and 7%) in a polythene bag The incubation period of all samples was 5 days at room temparature. The treatments were T_0,T_1 T_2, T_3 and T_4.

Fermentation of roughage mixture

Roughage mixture was prepared at a 1:3 (Rice straw: Sugarcane bagasse) ratio. Fermentation was carried out with 50 g roughage mixture, 50 ml warm water (100% of substrate) and dry yeast (0, 1, 3, 5, and 7%) in a polythene bag. The incubation period of all samples was 5 days at room temparature. The treatments were T_0,T_1 T_2, T_3 and T_4.

Nutritional analysis of fermented feed

Chemical composition (CP, CF, EE & NFE) of fermented feed was analyzed by the AOAC (2011) method on DM basis. *In-vitro* gas (CH_4) and Total phenol (TP) was determined by Khan and Chaudhry (2010) method. Major mineral (Ca & P) was determined by Atomic Absorption Spectrophotometry method.

Prepared fermented samples were dried in an oven (Dewsil Stainless Steel Laboratory Oven, Model no.10411, India) for 24hr at 105°C.

a) Proximate analysis

Moisture

Moisture (%) = 100-DM (%)

Dry matter (DM)

DM (%) = Wt. of dry sample × 100 / Wt. of fresh sample (g)

Total ash (TA)

Total Ash (%) = Wt. of ash × 100/Wt. of sample (g)

Crude protein (CP)

The CP content was calculated by using following formula,

Nitrogen content of sample (%) =
0.1N × 0.014 × Titration value ×100/Wt. of sample (g)
Crude protein content (%) = Nitrogen content × 6.25

Crude fiber

Crude fiber (%) =
(Wt. of crucible + dried residue) - (Wt. of crucible + ash residue) ×100/Wt. of sample (g)

Ether extracts

The amount of fat and oil in the feed is represented by the crude fat (ether extract). The following formula was used to compute the ether extract.
Crude fat or Ether extract (%) = Wt. of fat × 100/Wt. of sample (g)

Nitrogen free extracts

Mathematical calculations are used to determine NFE. It is obtained by deducting the total number of nutrients percentages from 100.
NFE % = {100- (CF % + CP % + EE % + TA %)}

b) Measurement of Calcium

A pipette was used to put 20 ml of the diluted filtrate into a 50 ml volumetric flask, fill it to volume with water, and then mix it. Using an atomic absorption spectrometer, Ca was measured (AAS). Compared to the highest standard solution, the reading was greater. It was diluted heavily, for example, by adding 10 ml of filtrate to a 50 ml volumetric flask. To make the total volume of the 1:100 diluted HNO_3 and filtrate equal to 20 ml, 1:100 diluted HNO_3 was then added to the volumetric flask.

c) Measurement of Phosphorus

Diluted filtrate 5 ml (pipette) was transferred to a 50 ml volumetric flask. Approximately 30 ml water was added. A 10 ml ammonium molybdate solution was added to make volume with water and mix. After 15 minutes the absorbance was measured on a spectrophotometer at 890 nm. When the absorbance was higher than that of the highest standard solution, the procedure was repeated by using a smaller amount of filtrate. In that case 1:100 diluted HNO_3 was added to the volumetric flask to make the total volume of 1:100 diluted HNO_3 and filtrate equal to 5 ml. If the content of P is very high, it is necessary to dilute the filtrate further before the transfer to the 50 ml flask. A volumetric flask and pipette were used to create the dilution using water. After adding 5 ml of the diluted filtrate to the 50ml volumetric flask, 5 ml of the diluted HNO_3 (1:100) and approximately 30ml of water were also added. After adding 10 ml of ammonium molybdate, filling the 50 ml volumetric flask to volume with water, and waiting 15 minutes, the absorbance was measured at 890 nm.

d) Measurement of Phenol

The total phenolics were calculated using the Folin-Ciocalteu technique (Singleton and Rossi, 1965), as reported by Khan and Chaudhry, (2010). The total phenolic content of extracts was calculated by suspending 0.1 g of samples in 10 ml of 75% aqueous acetone in test tubes. Using a vortex mixer (Model-XH-C, China), the test tube contents were vortexed for 30 minutes. Aliquots (0.1 ml) of every ether extract were combined with 0.4 ml of water, 0.25 ml of Folin-Ciocalteu reagent, and 1.25 ml of sodium carbonate solutions (20%). A UV/vis Spectrophotometer (SP-MUV5100) was used to measure the absorbance after standing at room temperature for 40 minutes and reading it at a wavelength of 760 nm in the visible spectrum. All samples underwent triplicate analysis to determine the total phenolic content of the extracts. The results were calculated using tannic acid as the standard, and they were reported as mg of tannic acid equivalent per gram of material on a dry mass basis.

e) Measurement of Tannins

Tannins were calculated by Khan and Chaudhry, (2010) method. Using polyvinyl pyrrolidone (PVP), Tannins were measured as the difference between phenolics in the extract before and after tannin removal. A sample of 0.1g PVP was placed in a test tube. An aliquot (1 ml) of each extract from the prior stage was added, along with 1 ml of water. 30 minutes were spent incubating the mixtures at 4°C after vortex mixing. The mixtures were then centrifuged once more for 3 minutes to complete the second precipitation stage. For a second precipitation process, the supernatant was centrifuged once more for three minutes. A second collection of supernatant was made. Each supernatant obtained last was divided into aliquots (0.1 ml), which were then combined with 0.4 ml of water, 0.25 ml of Folin-Ciocalteu reagent, 1.25 ml of sodium carbonate solutions (20%), and the same procedures as indicated in the preceding sections. The value of total was subtracted from total phenolic to get the total tannin content.

f) Cellulase enzyme assay method

The Mandels and Weber, (1969) method was used to measure cellulase enzyme (CMCase) activity. A test tube containing 1.8 ml of 1% CMC produced in 50M sodium citrate buffer (pH 4.8) received 0.2 ml of culture supernatant before being incubated at 60°C for 30 minutes. A 3.0 ml of dinitrosalicylic acid (DNS) reagent was added to the reaction to stop it, and the reaction tubes were then submerged in a water bath heated to 100ºC for five minutes and stabilized by adding 1.0 ml of Rochelle salt solution (40 g Rochelle salt in 100 ml distilled water). At a specific wavelength of 575 nm, the absorbance/optical density (OD) was measured against

a blank of 50M sodium citrate buffer. One mole of glucose liberated per milliliter of enzyme per minute was used to express one unit of CMCase activity.

In a test tube with a loose cover, 3 ml of the DNS reagent and 3 ml of the glucose sample were combined. The red-brown color of the mixture was created by heating it at 100°C for 15 minutes. The color was then stabilized with 1 cc of a 40% potassium sodium tartarate (Rochelle salt) solution. A spectrophotometer was used to measure the absorbance at 575 nm after the sample had cooled to room temperature in a cold water bath.

g) Measurement of Methane gas (CH_4) and Metabolizable energy (ME)

Collection of total gas (TG)

The gas created during the *in vitro* test was collected using a calibrated plastic gas syringe. At the completion of each set of incubation times, the fermentation parameters were observed. To monitor the positive pressure produced by the gas buildup in the syringe's headspace at room temperature and allowing the gas to flow within a syringe barrel, a needle channel linked to the syringe was extended into the sealed fermentation container. The total gas (TG) produced in ml was measured as the pressure at which the volume of trapped gas inside the barrel was measured.

CO_2 and CH_4 measurement

Lime-water was prepared for the measurement of CH_4 and CO_2. The TG contained gas syringe sink into the lime-water jar and backward pressure of syringe take the lime water into the syringe tube where the CO_2 itself reacts with the lime and disappear. The rest of the gas in the syringe tube indicates the amount CH_4 production in ml. Rest of this CH_4 amount subtracted from measured TG and this result indicates CO_2 production in ml (Mel et al., 2014).

In vitro incubation

Incubation was conducted at 39°C temperature in water bath. The duration was maintained up to 72 hr and gas production was recorded at 0, 1, 6, 12, 24, 36, 48 and 72 hr intervals, respectively.

Metabolizable energy (ME)

Total gas (TG) production was recorded after 72 hr of incubation. By using chemical composition and total gas production at 72 hr incubation (TG, ml), metabolizable energy (ME, MJ/kg DM) were calculated according to Menke and steingass (1988).

ME (MJ/kg)=2.20+0.1357 TG+0.0057 CP+0.000286 EE

ME=Metabolizable energy (MJ/kg DM); TG= Total gas production is expressed in ml/200mg DM; CP= Crude protein (g/kg DM); EE= Ether extract (g/kg DM).

Statistical analysis

The data were arranged according to one way ANOVA and treatment variation was determined with Minitab19 statistical software program. Nutritional composition of fermented roughages with different yeast levels were considered.The level of significance was determined at $p<0.05$.

Results

Nutritional composition of fermented rice straw with different yeast level

Table1 shows the nutritional composition of rice straw incubated with yeast for 5 days. Yeast fermentation increased the CP content (39.26%),. The reverse result was in CF, the reduction of CF and EE was 24.45% and 29.75%, respectively due to the addition of yeast. The addition of yeast increased the TA (6.86%), NFE (13.53%) levels. Yeast fermented rice straw was found rich in CP, and TA content. The tannin (mg/g DM) content decreased (41.17%) significantly with the increment of yeast dose. The TP (mg/g DM) increased (25.28%) with the increased yeast dose. Results showed that fermentation significantly ($p<0.001$) decreased (31.32%) gas production (ml/200 mg DM) that was lower in T_4 treatment. The CH_4 gas production decreased (8.78%) that was significantly higher ($p<0.001$) in T_0 treatment. The ME (MJ/kg DM) content of fermented rice straw decreased (17.30%) with yeast dose up gradation. Result showed that Ca and P increased by 38.72% and 31.25% respectively after yeast fermentation.

Nutritional composition of fermented sugarcane bagasse with different yeast level

Table 2 shows that dry yeast fermentation improved the nutritional composition of sugarcane bagasse. Fermentation reduced the CF 25.15% and EE 62.5%in fermented bagasse. Fermentation increased the NFE 14.77% content of bagasse. Yeast fermentation also increased the TA content 48.60% of bagasse in 7% yeast addition. Yeast increased the CP (78.40%), TA (48.60%), and NFE (14.77%) levels significantly ($p<0.001$). Result showed that Ca and P increased by 42.0% and 28.57% respectively after yeast fermentation. The tannin (mg/g DM) content decreased (93.33%) significantly up to 7% addition of yeast. Fermentation increased 38.70% TP (mg/g DM) content of sugarcane bagasse. The result showed the TG production (ml/200 mg DM) *in vitro* trial declines (29.66%) up to 7% yeast addition. The CH_4 gas production declines (39.97%) up to 7% yeast addition. The ME (MJ/kg DM) content decreased (13.86%) in fermented bagasse.

Table 1. Nutritional composition of fermented rice straw with different yeast level

Parameter	Treatment					SEM	p value
	T_0	T_1	T_2	T_3	T_4		
CP (%)	3.82^{e}	4.42^{d}	5.0^{c}	5.14^{b}	5.32^{a}	0.03	<0.001
CF (%)	34.02^{a}	30.23^{b}	27.32^{c}	27.02^{d}	25.7^{e}	0.01	<0.001
EE (%)	1.21^{a}	1.2^{a}	1.02^{b}	0.97^{c}	0.85^{d}	0.01	<0.001
TA (%)	16.02^{e}	16.51^{d}	16.91^{c}	17.22^{a}	17.12^{b}	0.01	<0.001
NFE (%)	44.93^{e}	47.64^{d}	49.75^{b}	49.55^{c}	51.01^{a}	0.04	<0.001
ME (MJ/kg DM)	6.01^{a}	5.87^{b}	5.78^{c}	5.37^{d}	4.97^{e}	0.02	<0.001
Cellulase (unit/ml)	0.010^{e}	0.010^{d}	0.011^{c}	0.012^{b}	0.013^{a}	0.02	<0.001
TP (mg/g DM)	0.87^{e}	0.90^{d}	1.01^{c}	1.04^{b}	1.09^{a}	0.01	<0.001
Tannin (mg/g DM)	0.51^{a}	0.41^{b}	0.4^{b}	0.34^{c}	0.30^{d}	0.01	<0.001
TG (ml/200 mg DM)	26.5^{a}	25.2^{b}	24.3^{c}	21.1^{d}	18.2^{e}	0.12	<0.001
CH_4 (ml/200 mg DM)	19.0^{a}	18.4^{b}	18.1^{c}	17.9^{d}	17.33^{e}	0.06	<0.001
Ca (g/kg)	1.73^{e}	1.80^{d}	1.94^{c}	2.0^{b}	2.4^{a}	0.11	<0.001
P (g/kg)	0.8^{c}	0.91^{b}	0.99^{b}	1.02^{a}	1.05^{a}	0.05	<0.001

T_0,RS; T_1, RS +1% dry Yeast; T_2, RS +3% dry yeast; T_3, RS +5% dry yeast; T_4, RS +7% dry yeast; RS, Rice straw; TA, Total ash; CP, Crude protein; CF, Crude fiber; EE, Ether extract; NFE, Nitrogen free extract; TP, Total phenol; ME, Metabolizable energy; TG, Total gas; CH_4, Methane; Ca, Calcium; P, Phosphorus; SEM, Standard error of mean.

Table 2. Composition of fermented sugarcane bagasse with different yeast level

Parameter	Treatment					SEM	p value
	T_0	T_1	T_2	T_3	T_4		
CP (%)	1.76^{d}	2.01^{c}	2.31^{b}	2.80^{b}	3.14^{a}	0.01	<0.001
CF (%)	43.05^{a}	41.22^{b}	38.12^{c}	37.41^{d}	32.22^{e}	0.02	<0.001
EE (%)	0.8^{a}	0.74^{b}	0.68^{bc}	0.42^{c}	0.30^{d}	0.01	<0.001
TA (%)	5.39^{e}	6.11^{d}	7.15^{c}	7.42^{b}	8.01^{a}	0.03	<0.001
NFE (%)	49.0^{e}	49.92^{d}	51.74^{c}	51.95^{b}	56.24^{a}	0.02	<0.001
ME (MJ/kg DM)	4.76^{a}	4.63^{b}	4.51^{c}	4.31^{d}	4.10^{e}	0.01	<0.001
Celllase(unit/ml)	0.007^{e}	0.007^{d}	0.008^{c}	0.010^{b}	0.010^{a}	0.01	<0.001
TP (mg/g DM)	0.31^{e}	0.34^{d}	0.39^{c}	0.40^{b}	0.43^{a}	0.01	<0.001
Tannin (mg/g DM)	0.15^{a}	0.14^{a}	0.04^{b}	0.03^{b}	0.01^{b}	0.01	<0.001
TG (ml/200 mg DM)	18.1^{a}	17.6^{b}	16.1^{c}	14.3^{d}	12.73^{e}	0.06	<0.001
CH_4 (ml/200 mg DM)	15.16^{a}	14.16^{b}	11.0^{c}	10.1^{d}	9.1^{e}	0.06	<0.001
Ca (g/kg)	0.5^{d}	0.58^{c}	0.60^{b}	0.66^{a}	0.71^{a}	0.08	<0.001
P (g/kg)	0.63^{d}	0.66^{c}	0.70^{b}	0.78^{a}	0.81^{a}	0.13	<0.001

T_0= SB, T_1= (SB +1% dry yeast), T_2= (SB +3% dry yeast), T_3= (SB +5% dry yeast) , T_4= (SB +7% dry yeast), SB=Sugarcane bagasse,TA=Total ash, CP=Crude protein, CF=Crude fiber, EE=Ether extract, NFE=Nitrogen free extract, TP=Total phenol, ME=Metabolizable energy, TG=Total gas, CH_4= Methane, Ca= Calcium, P= Phosphorus, SEM=Standard error of mean.

Nutritional composition of fermented mixed roughage (Rice straw: Sugarcane bagasse; 1:3) with different yeast level

Table 3 shows the nutritional composition of the roughage mixture (Rice straw: sugarcane bagasse) which was prepared at a 1:3 ratio and fermented by dry yeast for up to 5 days incubation period. Results revealed that dry yeast fermentation increased 29.91% CP content. The reverse result was in CF. Reduction of CF and EE was 26.71% and 34.61% respectively. Meanwhile, NFE content increased 16.91% with yeast dose. Yeast increased the CP (29.91%), TA (21.12%) and NFE (16.91%) levels significantly. Yeast fermented roughage mixture was found rich in CP and TA content. The Tannin (mg/g DM) content of the mixture decreased 36.67% significantly with the increment of yeast dose. Fermentation increased 24.56% TP (mg/g DM) content of roughage mixture with yeast level. The ME (MJ/kg DM) content of fermented roughage mixture was decreased by 13.76%. The result showed that TG production (ml/200 mg DM) *in vitro* trial declined 29.78%. The CH_4 gas (ml/200 mg DM) production significantly decreased 46.56%. The table showed that dry yeast also improved the mineral Ca and P (g/kg) content of the roughage mixture 35.53 % and 34.0% respectively.

Table 3. Nutritional composition of fermented mixed roughage (Rice straw + Sugarcane bagasse) with different yeast level

Parameter	Treatment					SEM	p value
	T_0	T_1	T_2	T_3	T_4		
CP (%)	3.71^{e}	3.86^{d}	4.01^{c}	4.22^{b}	4.82^{a}	0.01	<0.001
CF (%)	38.89^{a}	35.83^{b}	32.31^{c}	30.12^{d}	28.50^{e}	0.01	<0.001
EE (%)	1.56^{a}	1.42^{b}	1.26^{c}	1.11^{d}	1.02^{e}	0.01	<0.001
TA (%)	8.85^{e}	9.2^{d}	9.82^{c}	10.41^{b}	10.72^{a}	0.01	<0.001
NFE (%)	46.99^{e}	49.69^{d}	52.6^{c}	54.14^{b}	54.94^{a}	0.04	<0.001
ME (MJ/kg DM)	4.87^{a}	4.74^{b}	4.61^{c}	4.39^{d}	4.20^{e}	0.01	<0.001
Cellulase (unit/ml)	0.008^{e}	0.009^{d}	0.010^{c}	0.010^{b}	0.011^{a}	0.01	<0.001
TP (mg/g DM)	0.57^{e}	0.60^{d}	0.65^{c}	0.68^{b}	0.71^{a}	0.01	<0.001
Tannin (mg/g DM)	0.3^{a}	0.29^{b}	0.27^{c}	0.22^{d}	0.19^{e}	0.01	<0.001
TG (ml/200 mg DM)	18.13^{a}	17.06^{b}	16.1^{c}	14.3^{d}	12.73^{e}	0.06	<0.001
CH_4 (ml/200 mg DM)	15.16^{a}	10.1^{b}	9.4^{c}	9.0^{d}	8.1^{e}	0.06	<0.001
Ca (g/kg)	1.21^{e}	1.33^{d}	1.41^{c}	1.56^{b}	1.64^{a}	0.03	<0.001
P (g/kg)	1.0^{e}	1.09^{d}	1.16^{c}	1.23^{b}	1.34^{a}	0.01	<0.001

T_0=RM, T_1=(RM+1% dry Yeast), T_2=(RM +3% dry yeast), T_3= (RM +5% dry yeast), T_4= (RM +7% dry yeast), RM= Roughage mixture, TA=Total ash, CP=Crude protein, CF=Crude fiber, EE=Ether extract, NFE=Nitrogen free extract, TP=Total phenol, ME=Metabolizable energy, TG=Total gas, CH_4= Methane, Ca= Calcium, P= Phosphorus, SEM=Standard error of mean

Discussion

Protein

The ability of *S. cerevisiae* to produce extracellular enzymes (protein) is linked to the nitrogen enrichment of the fermentation medium (Oboh and Akindahunsi, 2003). Microorganisms use carbohydrates as an energy source during fermentation and produce carbon dioxide as a byproduct. This allows the nitrogen in the fermented product to be concentrated, which raises the mass's proportion of protein (Nasseri et al., 2011). This may be due to the production of microbial proteins including enzymes, hydrolyzed peptides, and other nitrogenous microbial components like chitin (Oseni and Akindahunsi, 2011). In general, carbohydrates are used by microbes as a source of energy, and through intermediate metabolism, they are bio-converted into microbial proteins (Rajesh et al. 2010). Sharma & Kumawat, (2021) demonstrate that some bacteria are capable of fixing nitrogen from the atmosphere. Even if the amount of nitrogen fixed may be minimal, it is crucial for microbial development and the formation of metabolites like amino acids and other compounds. It is most likely because concentrated enzymes make it easier for starch and cellulose to break down, which raises the protein concentration.

Fiber

The fermenting organisms has the ability to metabolize the fiber could be the cause of the overall decrease in the fiber content. It may also be a result of the fiber's enzymatic degradation during fermentation by microbes which use the fiber as a carbon source (Ojokoh and Bello, 2014). Microorganisms that hydrolyze and metabolize insoluble polysaccharides may secrete extracellular enzymes, which may be the cause of the decrease in crude fiber content of fermented products. Another reason is that bacteria like *L. manufacture* the enzyme ß-D glucosidase. The polysaccharide chains' terminal, nonreducing portions can be hydrolyzed by planta-rum and this enzyme (Minnaar et al., 2017). The total crude fiber content of the fermented feeds typically declines because the soluble dietary fiber content of the product is often reduced more than the insoluble dietary fiber content (Comino et al., 2018). The decrease in fiber content following fermentation is a sign of softening of fibrous tissues and higher digestibility as a result of activities of microorganisms recognized for the bioconversion of carbohydrates and lignocellulose into protein (Adegunloye&Oparinde, 2017).

Fat

The decrease in fat level may be explained by the fact during fermentation; microbes used fat as an energy source for their metabolic processes. Additionally, the oxidation process that could occur during fermentation may be the cause of the decrease in fat content (Li et al., 2020). It might also result from the fermenting organism's metabolism of fatty acids and glycerol, which improves flavor, fragrance, and texture (Ojokoh et al., 2014).Yeast uses fat as an energy source to build cell biomass as a result the amount of fat in the fermented products would be decreased. The biochemical and physiological processes that occur during fermentation demand energy and some of the lipids in the feeds were used to produce energy, which may explain why the amount of fat in the fermented feed (concentrate) decreased during the study (Afify et al., 2011).

Energy

The present research showed energy (ME) content of the feed was reduced by (7.39-17.30)% due to yeast *(S.*

cerevisiae) fermentation (Rice straw 17.30%, sugarcane bagasse 13.86%, mixed roughage 13.75% and mixed concentrate 7.39%). The *S. cerevisiae* strain has the capacity to hydrolyze complex carbohydrates into sugars, which are employed as a carbon source in the synthesis of high-protein microbial biomass, may account for the drop in energy content during fermentation (Akintomide and Antai, 2012). It is possible that yeast's usage of fermentable sugars for development, energy, and other metabolic processes is what causes the fall in carbohydrate content with fermentation (Ojokoh et al., 2013). Free sugars and starches found in substrate were used by microorganisms an energy source during fermentation (Gopalan et al., 1999).

Ash

In addition to being a rich source of minerals, yeasts can also add up to 10% of ash to the fermented mass, depending on the species and strain (Dobrzaski et al. 2008). The increase in ash content may be linked to the fermentation process's loss of organic matter and accumulation of inorganic materials, which is facilitated by the actions of enzymes and microorganisms (Uvere et al., 2010). The proportional rise in the unchanged components of the fermented product, particularly the fiber and ash contents, may be the cause of the increase in crude ash observed because of the dry matter loss during fermentation.

Phenol

The action of microbes, which use the soluble and fermentable fiber for their growth, may be the reason for the increased total phenolic values in the fermented sample. Microorganism released "mechanically imprisoned" phenolic chemicals in the polymeric structures of the fiber (Balli et al., 2020). Microbial enzymes are released during the fermentation process, which results in the production of plant compounds like flavonoids, tannins, alkaloids, and phenylpropanoids that are more readily available (Messen and Vuyst, 2002). The easy phenolic conversion and depolymerization of high molecular weight phenolic compounds are both facilitated by the presence of microbes during fermentation (Othman et al., 2009).

Mineral (Ca & P)

Plant sources minerals have very low bioavailability because they are found complexed with non-digestible material such as cell wall polysaccharides as well as phytate (Torre et al., 1991). Their limited bioavailability is mostly caused by the intricate matrices in which these minerals are bound and trapped. One of the processing techniques used to release these complex minerals and increase their bioavailability is fermentation. Some fermented feeds included more calcium due to fermentation due to a reduction in the amount of phytates (Pranoto et al., 2013). Day and Morawicki, (2016) explained that the loss of dry matter during fermentation as microbial break down of protein and carbohydrate may be the cause of the increased mineral content.

Tannin

Tannins are a collection of water-soluble phenolic compounds with a wide range of chemical properties that bind to proteins to create soluble or insoluble complexes and change their structural and functional characteristics (Girard et al., 2018). The breakdown of tannin complexes such tannic acid-starch, tannin-protein, and tannin-iron complexes to release the bound nutrients might induce the reduction in tannin levels by hydrolyzing the polyphenolic components into simpler molecules (Obizoba and Atii, 1991). Through non-covalent or covalent interactions, the dietary proteins create complexes with phenolic substances. Both reaction processes have the potential to change the chemical makeup of proteins and phenolics they interact with, altering their biological, functional, and nutritional properties as well as the features of the final product (Zhang et al., 2020). As a result, the reduction may be attributed to the tannase enzyme, which can hydrolyze complex tannins, hydrolysable tannins, and condensed tannins (Kuddus, 2018).

Cellulase enzyme

Cellulase enzyme activity is acquired at the beginning of the fourth day of fermentation. As fermentation progresses, enzyme activity rises. However, on the fifth and sixth days, enzyme activity dropped. Enzyme fermentation activity is initially still relatively modest. Enzyme activity rises with longer fermentation times and falls when nutrients are scarce (Gunam et al., 2010). Enzymes are typically produced in the post exponential period by spore-forming organisms. The mold has therefore been in that phase when the enzyme activity is high (Singhania et al., 2010). Different optimum fermentation times for the generation of cellulase enzyme due to variations in the qualities of the medium, the kinds of microorganisms used, the concentrations of nutrients, and the physiological circumstances of the process used (Ojokoh et al., 2013).

Methane gas (CH_4)

With the fermentation process being changed in a way that lessens CH_4 generation, they brought it back to *S. cerevisiae* capacity to influence H_2 metabolism in the rumen. Gas production depends on nutrient availability for rumen microorganisms (Mahala and Fadel Elseed, 2007). The rumen produces gases that are mostly made up of hydrogen, carbon dioxide, and methane as a result of the fermentation of dietary carbohydrates to

acetate, propionate, and butyrate. However, compared to the fermentability of carbohydrates, protein fermentation results in a comparatively low gas production (Makkar et al., 1996). *S. cerevisiae* has the capacity to reduce methane and ammonia generation as well as increase fermentation efficiency, which can help to lower greenhouse gas emissions (Hristov et al., 2013).Additionally, reducing the rumen's capacity to degrade proteins and produce ammonia (Mao et al., 2013) has the potential to reduce the animal's overall nitrogen excretion, which would help reduce ammonia emissions from cow manure. The creation of CO_2 and H_2, which are byproducts of acetate synthesis during carbohydrate fermentation, may have contributed to the lower production of total CH_4 in the yeast treatments (Gong et al., 2013).

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Conclusion

The objectives of this study were to improve the nutrient composition of two poor-quality roughages (Rice straw and Sugarcane bagasse) by yeast (*S.cerevisiae*) fermentation.Yeast *(S. cerevisiae*) fermentation can improve the protein content of rice straw up to 39.26% and sugarcane bagasse by up to 78.40%. Fermentation is one feed improvement technology that can improve the nutritional content of feed for small ruminants.

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